# Transcriptional profile of an altered purine metabolism in skeletal muscle of *Hdh*Q150 mouse model

## Profil transkrypcyjny w zaburzonym metabolizmie puryn w mięśniu szkieletowym mysiego modelu *Hdh*Q150

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**Wprowadzenie.** Choroba Huntingtona (HD) jest nieuleczalną chorobą neurodegeneracyjną powodowaną wydłużeniem łańcucha poliglutaminowego huntingtyny (HTT). Istnieje również obwodowe uszkodzenie w przebiegu HD: mięśnie szkieletowe są poważnie uszkodzone co prowadzi do zaniku i upośledzenia ich funkcji, wykazanego zarówno w warunkach przedklinicznych, jak i w klinicznych. W mysim modelu R6/2 odpowiadającym postaci młodocianych HD wykazano ostatnio obecność zmienionych transkryptów odpowiedzialnych za metabolizm puryn w różnych typach mięśni szkieletowych.

**Cel.** Walidacja zmian transkrypcji kluczowych enzymów biorących udział w metabolizmie puryn w różnych typach mięśni szkieletowych w mniej agresywnym mysim modelu *Hdh*Q150.

**Materiał i metody.** Wykorzystano 22-miesięczne myszy *Hdh*Q150 jako źródło różnych typów mięśni szkieletowych, aby określić zmiany transkrypcji za pomocą qPCR Taqman.

**Wyniki.** Zidentyfikowano liczne geny o zmienionej aktywności w mięśniach szkieletowych myszy *Hdh*Q150 związane z zaburzeniami gospodarki energetycznej oraz metabolizmem puryn.

**Wnioski.** Przedstawiono pierwszą analizę modyfikacji transkrypcji odzwierciedlającej zmiany w homeostazie energetycznej i metabolizmie puryn mięśni szkieletowych starszego mysiego modelu *Hdh*Q150. Również przedstawiono liczne zmiany transkrypcji związane ze zmianami patologicznymi obserwowanymi w mysim modelu HD.

**Słowa kluczowe:** *choroba Huntingtona, zanik mięśni szkieletowych, metabolizm puryn, biomarkery, model mysi*

**Introduction.** Huntington's disease (HD) is a fatal neurodegenerative disorder, caused by a polyglutamine expansion in the huntingtin protein (HTT). HD has a peripheral component to its pathology: skeletal muscles are severely affected, leading to atrophy and malfunction in both pre-clinical and clinical settings. We recently reported a number of altered transcripts related to the overall purine metabolism in different types of skeletal muscle, in the very aggressive R6/2 mouse model that replicates a juvenile form of HD in humans.

**Aim.** To validate transcriptional alterations of the key enzymes involved in the purine metabolism in different types of skeletal muscles in the less aggressive model of aged *Hdh*Q150 mice.

**Material & methods.** We used 22 months old *Hdh*Q150 mice as a source of different types of skeletal muscle, in order to establish transcriptional changes by employing Taqman-qPCR.

**Results.** We identified a number of genes related to energy imbalance and purine metabolism, that are deregulated in the skeletal muscles of these *Hdh*Q150 mice.

**Conclusions.** We report the first transcriptional signature that represents changes in energy homeostasis, and an altered purine metabolism, in the skeletal muscles of an aged *Hdh*Q150 mouse model. Consequently, we report a number of transcript alterations that are linked to HD muscle pathology.

**Key words:** *Huntington's disease, skeletal muscle atrophy, purine metabolism, biomarkers, mouse models*



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## Introduction

Huntington's disease (HD) is an inherited, multi-systemic monogenic neurodegenerative disorder [1]. It is caused by a CAG-repeat expansion within the Exon-1 of the huntingtin gene (*HTT*), that translates into a polyglutamine stretch (polyQ) in *HTT* protein. The mutation leads to gain or loss of function of the *HTT* protein that is normally expressed at high levels in a wide variety of mammalian tissues [2]. HD is characterised by motor dysfunction, cognitive decline and a progressive dementia, with the first symptoms typically occurring in midlife [for review see 3]. In addition, HD patients develop a pronounced muscle wasting, leading to malfunction, that has been widely

described in both pre- and clinical settings [reviewed in 4, 5]. We have recently described physiological and functional changes in the skeletal muscles of two well-established HD mouse models, namely R6/2 and *Hdh*Q150 [6]. These symptomatic HD mouse models had substantial alterations in energy equilibria in various skeletal muscles, accompanied by decreased levels of the phosphocreatine/creatine ratios, as well as lower ADP and AMP levels. In addition, the total pools of the adenine nucleotides were also consistently lower in HD mice [6]. Consequently, we recently reported a number of altered transcripts related to the overall purine metabolism in different types of skeletal muscle in the very aggressive R6/2 mouse model; this model replicates a juvenile version of HD in humans [7]. We previously demonstrated altered purine metabolism and energy imbalance are also altered in the milder *Hdh*Q150 mouse model [6]. Our current study determines transcriptional alterations of key enzymes involved in purine metabolism in different types of skeletal muscles, in aged HdhQ150 mice. This allows us to identify potential biomarkers that reflect skeletal muscle wasting in an HD mouse model that mirrors adult onset HD in humans.

## Aim.

To validate transcriptional alterations of the key enzymes involved in the purine metabolism in different types of skeletal muscles in the less aggressive model of aged *Hdh*Q150 mice.

## Materials and methods

### *Mouse maintenance and genotyping*

The *Hdh*Q150 HD mouse line was bred and genotyped as previously described [6]. All experimental procedures were conducted under a project license from the Home Office, UK, and approved by the Animal Welfare and Ethical Review Body of Imperial College London. Experimental groups included the *Hdh*Q150 mouse model at 22 months of age (N=6) and their littermates (N=6). All animals had unlimited access to water and breeding chow (Special Diet Services, Witham, UK), and housing conditions and environmental enrichment were as described previously [6].

### *RNA extraction and Taqman real-time PCR expression analysis*

Total RNA from skeletal muscles (EDL (Extensor Digitorum Longus), SOL (Soleus), TA (Tibialis Anterior) and G/P (Gastrocnemius and Plantaris complex)) was extracted with a mini-RNA kit (Qiagen, UK), according to the manufacturer's instructions. The reverse transcription reaction was performed using MMLV superscript reverse transcriptase (Invitrogen, USA) and random hexamers (Sigma, USA), as described in an earlier study [8]. All Taqman qPCR reactions were performed with a LightCycler® 480 Instrument (Roche), as described previously [7]. Following Taq-man assays from Thermo Fisher Scientific were used in this study *Adsl* (Mm_00507759_m1); *Adssl1* (Mm_00475814_m1); *Gart* (Mm_00599836_m1); *Ppat* (Mm_00549096_m1); *Aprt* (Mm_04207857_g1); *Ak1* (Mm_00445475_m1); *Gmpr* (Mm_00499393_m1); *Impdh2* (Mm_00496156_m1); *Ada* (Mm_00545720_m1); *Adk* (Mm_00612772_m1); *Nt5e* (Mm_00501910_m1); *Ampd3* (Mm_00477495_m1); *Entpd2* (Mm_00515450_m1); *Pnp* (Mm_00840006_m1); *Xdh* (Mm_00442110_m1); *Prkaa1* (Mm_01296700_m1); *Pdk4* (Mm_01166879_m1); *Hk2* (Mm_00443385_m1). mRNA copy number was determined in triplicate for each RNA sample by comparison with the geometric mean of three endogenous housekeeping genes (Primer Design, UK), as described [8]. Stable housekeeping genes for qPCR profiling of various skeletal muscles for HD mouse models were determined using the Primer Design geNorm™ Housekeeping Gene Selection Mouse Kit with PerfectProbe™ software. We used following housekeeping genes: *Ubc*, *B2m* and *18S*.

All Taqman qPCR values were normalized to the geometric mean of three housekeeping genes. Error bars are ± SEM (N=6). Student's t test: *p<0.05, **p<0.01; ***p<0.001.

### *Statistical analysis*

Values were presented as mean ± SEM. The statistical analysis was performed using paired Student t tests (Excel) or One-Way Anova SPSS (IBM). A p-value of 0.05 was considered as a significant difference.

## Results

We previously reported the transcriptional signature related to energy imbalance and purine metabolism in the different types of skeletal muscle of R6/2 transgenic mouse model, that represents the fully symptomatic stage of a rapid-onset form of the disease [7]. However, the genetic basis of HD is more precisely recapitulated in so called knock-in models, like *Hdh*Q150, which possesses an elongated CAG repeat that has been inserted into the mouse Htt locus [9, 10]. In addition, this model develops a more slowly progressing phenotype that mirrors the adult version of HD in humans. Similarly to our earlier report, we focused on different muscles including Extensor Digitus Longum (EDL), Soleus (Sol), Tibialis Anterior (TA) and Gastrocnemius and Plantaris complex (G/P).

We found that the transcript levels of two genes involved in the purine nucleotide cycle (PNC) were significantly down-regulated. Adenylosuccinate lyase

(*Adsl*, Fig. 1A) and Adenylosuccinate lyase 1 (*Adlssl1*, Fig 1B) were reduced by approximately 50% in each type of skeletal muscle. In contrast, we found that Phosphoribosylglycinamide formyltransferase (*Gart*) was significantly up-regulated (on average 2-fold) in each type of skeletal muscle (Fig. 1C). Amidophosphoribosyltransferase (*Ppat*) mRNA was significantly reduced (Fig. 1D) while Adenine phosphoribosyltransferase (*Aprt*) transcripts remained unchanged (Fig 1E).

Next, we examined the transcriptional changes of genes involved in the conversion of purine nucleotides. We found that mRNA levels of Adenylate kinase 1 (*Ak1*; an enzyme that catalyses the terminal phosphate group between ATP and AMP) were significantly down-regulated in each type of skeletal muscle examined (Fig. 2A). The transcript levels of Inosine monophosphate dehydrogenase 2 (*Impdh2*) (catalyzes the conversion of IMP – Inosine 5'-phosphate to XMP – Xanthosine 5'-phosphate), were significantly up-regulated, by 50% (Fig. 2C). The transcript levels of Guanosine monophosphate reductase (*Gmpr*; maintains the intracellular balance of adenine and guanine nucleotides) remained unchanged (Fig. 2B).

Subsequently, we established the transcriptional changes of selected genes that are believed to be involved in adenosine metabolism. The mRNA levels of *Ada* (Adenosine deaminase) (Fig. 3A), *Adk* (Adenosine kinase) (Fig. 3B) and *Nt5e* (Ecto-5'-nucelotidase) (Fig. 3C) remained unchanged.

We next examined the transcriptional signature of genes involved in purine metabolism and their degradation. We found a significant up-regulation of the *Ampd3* (Adenosine monophosphate deaminase 3) specifically in the fast-type muscles like EDL (Fig. 4A), up to 6-fold. There was a significant down-regulation across tested conditions of *Entpd2* (Ectonucleoside triphosphate diphosphohydrolase 2; regulates ATP hydrolysis), specifically in fast-type skeletal muscles,

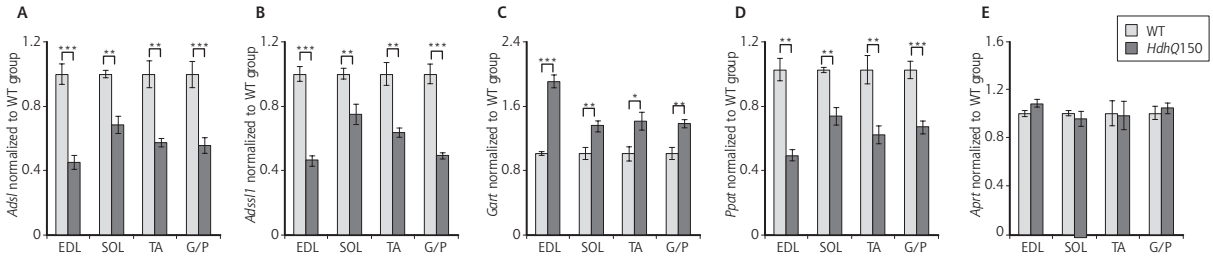


Fig. 1. Transcriptional alteration of genes involved in *de novo* purine synthesis and salvage pathway

Ryc. 1. Zmiany poziomu transkrypcji genów uczestniczących w syntezie puryn *de novo* ratunkowych szlaków metabolicznych

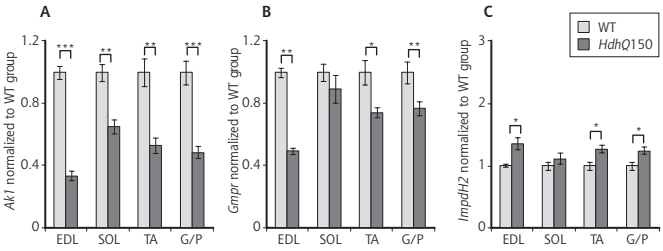


Fig. 2. Transcriptional changes of genes engaged in conversion of adenine nucleotides

Ryc. 2. Zmiany poziomu transkrypcji genów zaangażowanych w konwersję nukleotydów adeninowych

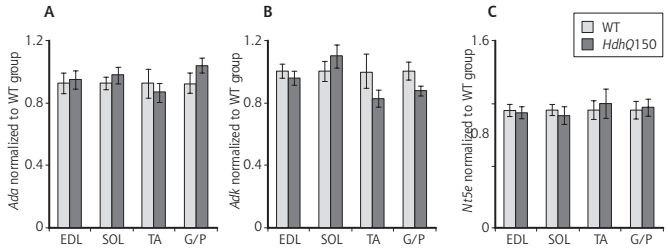


Fig. 3. Transcriptional levels of genes involved in adenosine metabolism

Ryc. 3. Zmiany poziomu transkrypcji genów zaangażowanych w metabolizm adenozyny

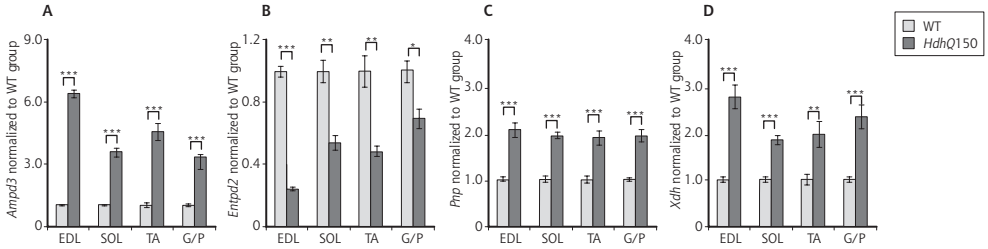


Fig. 4. Transcriptional changes of genes involved in purine metabolism and their degradation

Ryc. 4. Zmiany poziomu transkrypcji genów zaangażowanych w metabolizm puryn i ich degradację

Table I. A summary of altered genes related to purine metabolism and energy imbalance in HD mouse models
Tabela I. Podsumowanie zmian w genach związanych metabolizmem puryn i zaburzeniami metabolizmu energetycznego w mysim HD modelu

| Pathway /Szlak metaboliczny | Transcript name /Nazwa transkryptu | 12 weeks R6/2 /12-tygodniowe R6/2 | 22 months *Hdh*Q150 /22-miesięczne *Hdh*Q150 |
|---|---|---|---|
| *de novo* purine synthesis and salvage pathway /synteza puryn *de novo* i ratunkowe szlaki metaboliczne | *Adsl1* | down /obniżona | down /obniżona |
| | *Adssl1* | down /obniżona | down /obniżona |
| | *Gart* | up /podwyższona | up /podwyższona |
| | *Ppat* | unchanged /niezmieniona | down /obniżona |
| | *Aprt* | unchanged /niezmieniona | unchanged /niezmieniona |
| conversion of adenine nucleotides /konwersja nukleotydów adeniny | *Ak1* | down /obniżona | down /obniżona |
| | *Gmpr* | unchanged /niezmieniona | down /obniżona |
| | *Impdh2* | up /podwyższona | up /podwyższona |
| adenosine metabolism /metabolism adenozyny | *Ada* | unchanged /niezmieniona | unchanged /niezmieniona |
| | *Adk* | unchanged /niezmieniona | unchanged /niezmieniona |
| | *Nt5e* | unchanged /niezmieniona | unchanged /niezmieniona |
| purine metabolism and degradation /metabolism puryn i degradacja | *Ampd3* | up /podwyższona | up /podwyższona |
| | *Entpd2* | down /obniżona | down /obniżona |
| | *Pnp* | up /podwyższona | up /podwyższona |
| | *Xdh* | up /podwyższona | up /podwyższona |
| energy homeostasis /homeostaza energetyczna | *Prkaa1* | up/podwyższona | up /podwyższona |
| | *Pdk4* | up/podwyższona | up /podwyższona |
| | *Hk2* | down/obniżona | down /obniżona |

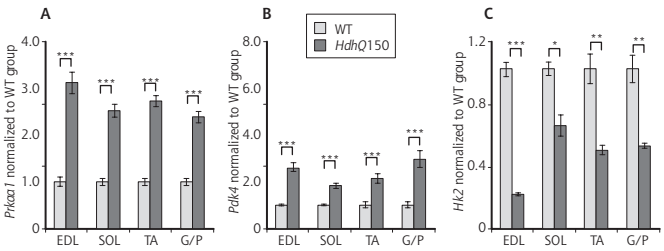


Fig. 5. Transcriptional levels of genes involved in energy homeostasis
Ryc. 5. Zmiany poziomu transkrypcji genów zaangażowanych w homeostazę energetyczną

such as EDL (Fig. 4B). Contrastingly, the mRNA levels of *Pnp* (Purine nucleoside phosphorylase) were significantly up-regulated across each muscle examined (Fig. 4C). We further examined the expression levels of mRNAs coding for enzymes involved in purine degradation pathways [11, 12] like *Xdh* (Xanthine dehydrogenase), and found them significantly up-regulated (Fig. 4D).

We also validated the expression levels of two genes involved in energy metabolism. *Prkaa1* (5'-AMP-activated protein kinase catalytic subunit alpha-1) mRNA levels were significantly up-regulated up to 3-fold, (Fig. 5A). Similarly, *Pdk4* (Pyruvate dehydrogenase, kinase isozyme 4) transcript levels were found to be uniformly up-regulated by 3-fold (Fig. 5B). The transcript levels of *Hk2* (Hexokinase 2) were significantly down-regulated (Fig. 5C). The data are summarised in Table I, comparing them to our previous study in R6/2 [7].

## Discussion

Huntington's disease is an autosomal dominant, neurodegenerative disorder for which there are currently only symptomatic treatments, reviewed in [3]. There is growing evidence that HD has a peripheral component contributing to its pathology, including skeletal muscle atrophy [4] and heart failure [13]. It has been shown that HD skeletal muscles develop an energy deficit and altered metabolism of purine nucleotides, spanning both fast and slow types of skeletal muscle in pre-clinical settings [6]. This might lead to the progressive impairment of the contractile characteristics of skeletal muscles in HD, because the pool of ATP, ADP and NAD+ was depleted in fast (EDL) and slow (Soleus) types of skeletal muscle, in both R6/2 and *Hdh*Q150 mouse models [6]. Consequently, we investigated and found an altered transcriptional profile of enzymes involved in energy imbalance, as well purine metabolism, in the aggressive R6/2 mouse model, which mimics a juvenile form of HD in humans [7]. Hence, the aim of this study was to identify the transcriptional signature of these key enzymes in the *Hdh*Q150 mouse model, that is believed to reproduce more precisely the adult onset of HD in humans. Similarly to our previous study in the R6/2 mouse model, we based our analysis on different types of skeletal muscle, chosen for their contrasting fiber compositions. However, this time we used older 22 month-old *Hdh*Q150 mice, reflecting the later onset of the disease phenotype in this model. It is well established that both HD mouse models (R6/2 and *Hdh*Q150) develop progressive skeletal muscle wasting. This might be due to muscle denervation (caused by loss of motor units function) or because of their inactivity [6]. We found a significant up-regulation of *Ampd3* transcript levels in aged skeletal muscle of *Hdh*Q150 mice, which is believed to be a marker of muscle disuse and muscle denervation [14]. A similar level of deregulation

was previously reported in the R6/2 mouse model. Moreover, we reproduced changes in gene expression of transcripts related to altered purine metabolism that tie in to our previous observations in R6/2 mice [7]. However, we noticed a relatively small number of discrepancies between these two HD mouse models. The transcript level of Amidophosphoribosyltransferase (*Ppat*) was significantly reduced in various types of skeletal muscle in the *Hdh*Q150 mice while it remained unchanged in the R6/2 mouse model as previously reported [7]. Similarly, Guanosine monophosphate reductase (*Gmpr*) mRNA levels were robustly reduced in the *Hdh*Q150 skeletal muscle, while they remained unchanged in the skeletal muscles of R6/2 mice. This might be explained by the age-related difference between these two HD mouse models. Overall, it is likely that these changes reflect skeletal muscle aging on top of an intrinsic effect of HD mutation.

## Conclusion

We report the first transcriptional signature that represents changes in energy homeostasis and an altered purine metabolism in the skeletal muscles of an aged *Hdh*Q150 mouse model. We believe that the current set of transcriptional alterations described in this second HD mouse model, strengthen their application as a useful set of HD biomarkers, both for tracking disease progression and for developing therapies.

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*Conflicts of interest statement: The authors declare no competing financial interests.*

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